AB The work describes a novel approach for sustained photobiological production of H2 gas via the reversible hydrogenase pathway in the green alga Chlamydomonas reinhardtii. This single-organism, two-stage H2 production method circumvents the severe O2 sensitivity of the reversible hydrogenase by temporally separating photosynthetic O2 evolution and carbon accumulation (stage 1) from the consumption of cellular metabolites and concomitant H2 production (stage 2). A transition from stage 1 to stage 2 was effected upon S deprivation of the culture, which reversibly inactivated photosystem II (PSII) and O2 evolution. Under these conditions, oxidative respiration by the cells in the light depleted O2 and caused anaerobiosis in the culture, which was necessary and sufficient for the induction of the reversible hydrogenase. Subsequently, sustained cellular H2 gas production was observed in the light but not in the dark. The mechanism of H2 production entailed protein consumption and electron transport from endogenous substrate to the cytochrome b6-f and PSI complexes in the chloroplast thylakoids. Light absorption by PSI was required for H2 evolution, suggesting that photoreduction of ferredoxin is followed by electron donation to the reversible hydrogenase. The latter catalyzes the reduction of protons to molecular H2 in the chloroplast stroma.
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AUTHOR(S): Melis, Anastasios [Reprint author]; Zhang, Liping; Forestier, Marc; Ghirardi, Maria L.; Seibert, Michael
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